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Light Scattering Investigation of Potato Amylopectin*

L. P. WITNAUER, *U. S. Department of Agriculture, Agricultural Research Service, Eastern Utilization Research Branch, Philadelphia 18, Pennsylvania*

F. R. SENTI, *U. S. Department of Agriculture, Agricultural Research Service, Northern Utilization Research Branch, Peoria 5, Illinois*

M. D. STERN, *Department of Chemistry, Temple University, Philadelphia, Pennsylvania*

INTRODUCTION

Compared to other physical methods, the light scattering method offers several advantages in the determination of the molecular weight of amylopectins. Osmotic pressure measurements on acetylated amylopectins of wheat,[1] sago,[1] Easter lily,[1] corn,[1,2] and tapioca[1,2] have indicated molecular weights for these derivatives ranging from 300,000 to 10 million. For molecular weights of this magnitude, accurate osmotic measurements are difficult to make, particularly in dilute solutions. Results of Kerr[2] *et al.*, indicating the dissociation of aggregates of corn amylopectin acetate in dilute chloroform solutions, demonstrate the desirability of data at concentrations below 0.1%. As will be reported in this paper, turbidities of potato amylopectin acetate solutions can be measured by the light scattering method at 0.02% concentration, and, because of the more favorable refractive index increment, scattering measurements can be made on even more dilute solutions of potato amylopectin in water. These concentrations are far below those practicable for sedimentation or diffusion measurements on amylopectin[3] solutions.

The light scattering method has the further advantage that alkaline solutions of amylopectin, as well as solutions at elevated temperatures, can be investigated conveniently. This is important in the study of molecular aggregation of amylopectin in water solutions for aqueous alkali has long been considered an excellent solvent for starch. Similarly, dissociation of aggregates would be expected to increase as the temperature of water solution is raised.

In an earlier investigation,[4] light scattering from potato amylopectin in water solution at angles of 45, 90, and 135° to the primary beam was

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measured. Assumption of spherical shape for the amylopectin molecule led to a molecular weight of 15 million, whereas interpretation of the scattering measurements on the basis of a random coil model gave a value of 32 million. Similar measurements on potato amylopectin using a visual photometer have been reported.[5] Assuming a random coil model, a molecular weight of 8 million was found; however, on standing 60 hours at 75° in water solution, this amylopectin degraded into particles about 1.4 million in molecular weight. A molecular weight, independent of assumptions concerning molecular shape, also was calculated[4] from the limiting specific turbidity obtained by the transmission method[6] from measurements made at several amylopectin concentrations and at wave lengths in the range 400 to 800 millimicrons. The value found, 27 million, was in fair agreement with the value calculated from the light scattering data on the assumption of a random coil model; the agreement, however, was probably fortuitous for no allowance was made for polydispersity in the latter computation. Molecular weights free from assumptions concerning molecular shape and polydispersity also can be obtained from light scattering data extrapolated to zero angle.[7,8] On the basis of such measurements, a thorough study has been made of potato amylopectin fractionated from whole starch by two different procedures. Observations have been made[9] both on amylopectin dispersed in water and on its acetate in a number of organic solvents. In the present paper, details of these studies and other observations on the polydispersity and solution properties of potato amylopectin will be given.

Experimental

Preparation of Amylopectin. Potato amylopectin (preparation I) was prepared from a high-grade commercial potato starch whose phosphoric acid was partly neutralized by ion exchange with a concentrated solution of potassium chloride.[10] A 2% starch paste (pH 6.0 to 6.5), heated in water at 90°, was dispersed by stirring vigorously for 15 to 30 minutes. The amylose fraction was separated by selective precipitation with Pentasol[11] and removed by two successive centrifugations, each of 15 minutes duration, in an International* centrifuge at 3500 r.p.m. The supernatant solution was then filtered through Corning sintered-glass filters of medium porosity. Pentasol was removed from the solution by vacuum distillation at 45 to 55°. The concentration of the resulting amylopectin solution was approximately 1.5%, representing a recovery of about 67% of the whole starch. Potentiometric titration of the amylopectin solution with iodine indicated an impurity of approximately 1.5% amylose. The amylopectin was maintained in solution to avoid formation of undispersible aggregates which might develop on precipitation. Addition of a trace of mercuric iodide protected the solution against bacterial action.

Another and considerably larger sample of potato amylopectin (preparation II) was prepared by autoclaving, at pH 6.0, a 2% paste of com-

* The mention of commercial products in this article does not constitute an endorsement by the U. S. Department of Agriculture.

mercial potato starch whose phosphoric acid was partly neutralized with calcium ion by ion exchange. The amylose fraction was precipitated with nitrobenzene[12] and separated by centrifugation. It was treated with cotton and the amylopectin recovered from the solution by precipitation with acetone. After two successive washings of the precipitate, it was allowed to air dry.

Optical Measurements. Light scattering measurements, at angles from 135 to 22° to the primary beam, were made with a Brice-Phoenix photometer[13] using a cylindrical scattering cell[14] with plane entrance and exit faces. Measurements were made on all solutions at wave length 436 mμ and, in addition, for some solutions, at 546 mμ.

The refractive increments of water solutions of amylopectin, as well as those of the partial acetate in various organic solvents, were determined in a differential refractometer[15] in the concentration range from 2 to 0.5%.

Clarification and Concentration of Solutions. Proper clarification of solutions prior to making the light scattering measurements is a critical requirement for reliable light scattering data. Because undispersed aggregates or particulate matter, such as dust, have large scattering factors, such particles must be removed from the solutions. Centrifugation, followed by filtration through Corning ultrafine sintered-glass filters, was found to give satisfactory results on amylopectin solutions as evidenced by the shape and reproducibility of the angular scattering curve, particularly in the low-angle region. Centrifugation was more effective in clarifying solutions relatively high in concentration (1 to 2%) compared to more dilute solutions. Probably the higher viscosity of the more concentrated solutions minimized the effect of thermal gradients and other disturbances during centrifugation and immediately thereafter.

Solutions of amylopectin I, approximately 1.5% in concentration, were centrifuged at 40,000 r.p.m. (~144,000 gravity, maximum sedimenting force) for 15 minutes in a Spinco Model L preparative ultracentrifuge. Contents of the centrifuge tube were removed with a hypodermic syringe without disturbing the sedimented material. This solution was centrifuged a second time for 15 minutes and the contents of the tube removed as before. The amount of material lost on centrifugation amounted to less than 5% of the dissolved amylopectin.

Weighed portions of the centrifuged amylopectin solution were diluted to a concentration of 10^{-4} to 10^{-6} g./ml., the concentration range suitable for light scattering measurements. The dilute solutions were filtered through sintered-glass filters of ultrafine porosity directly into the scattering cell. It was found advantageous not to make dilutions directly into the light scattering cell as anomalous scattering at low angles was usually observed even when extreme precautions had been taken.

Solutions of amylopectin II were clarified in a slightly different manner. Centrifugation of a 1.5% solution in the Spinco centrifuge at 17,000 r.p.m. (~60,000 gravity, maximum sedimenting force) for 1 hour was sufficient to permit ultrafine filtration of the solutions diluted for scattering measure-

ments. Less than 1% of the total amylopectin was removed on centrifugation.

The concentrations of water solutions of amylopectin were determined by evaporation of a weighed portion to partial dryness under a heat lamp, followed by drying in a vacuum oven at 110°. Approximately 4 hours was required to attain constant weight.

Fractionation of Amylopectin I. Two liters of a 1.5% water solution of amylopectin containing 0.1% NaCl was placed in a three-necked, three-liter, round-bottom flask, equipped with a mechanically operated stainless steel stirrer, and mounted in a controlled temperature bath at 31°. Ethyl alcohol was added dropwise to the solution with vigorous stirring until approximately 10% of the material separated. The required amount of alcohol had previously been determined by a solubility experiment conducted on a smaller scale. The bath was heated until the turbidity of the solution disappeared, and the system then allowed to cool slowly to 31°. After 48 hours the gelatinous precipitate was removed slowly through a stopcock at the bottom of the flask. Sufficient ethanol was added to the supernatant to separate another fraction, and the fractionation was continued until eight fractions were obtained. After removal of the eighth fraction the supernatant was concentrated to give the ninth fraction. The gelatinous precipitates of the fractions were dispersed in distilled water at 90° and 1% concentration, and the solutions dialyzed against distilled water to remove sodium chloride. Approximately 95% of the original amylopectin was recovered in the fractions.

Preparation of Amylopectin Acetate. Preparation of a partial acetate of amylopectin II was undertaken for it is common experience[16] that fully acetylated potato amylopectin is only partially soluble in any single organic solvent. Acetylation was carried out essentially by the method described by Carson and Maclay.[17] A 10-gram sample of amylopectin II was stirred at 60° with 200 ml. of formamide until dispersed. 160 ml. of pyridine was added to the formamide solution at room temperature. 60 ml. of acetic anhydride was added dropwise over a period of 15 minutes and the reaction was allowed to continue two additional hours at room temperature. During this period the solution developed a light yellow color. Any large aggregates present in the reaction mixture were removed by filtration through a sintered-glass filter of medium porosity. The amylopectin acetate was precipitated by pouring the reaction mixture into a large volume of water stirred in a Waring Blendor. After recovery by filtration, the precipitate was washed with water in the Waring Blendor. Remaining traces of acid were leached from the acetate by suspending it in 3 liters of water which was replaced at least once every day for a week. A fine white powder was obtained after air drying at room temperature. Different preparations of amylopectin acetate had acetyl contents in the range 38 to 40%. Amylopectin acetate obtained by the above procedure seemed to be completely soluble, without evidence of microgel, in several organic solvents. Acetone, chloroform, dioxane, nitromethane, and aceto-

nitrile dispersed the acetate readily without the application of heat. These solutions were clarified by centrifugation at 17,000 r.p.m. for 1 hour with less than 0.2% change in concentration.

RESULTS

Light Scattering Measurements on Water Solutions of Amylopectin I. Measurements on amylopectin I solutions at wave length 436 mμ, plotted as described by Zimm,[18] are given in Figure 1. The ordinate in Figure 1 and in succeeding figures is Hc/τ where H is the usual constant, c is the concentration in g./ml., and τ is the *apparent* turbidity of the solution minus that of the solvent. At zero angle ($\theta = 0$), where no reduction of the scattered light by intraparticle interference occurs, the apparent excess turbidity of the solution becomes the true excess turbidity. The

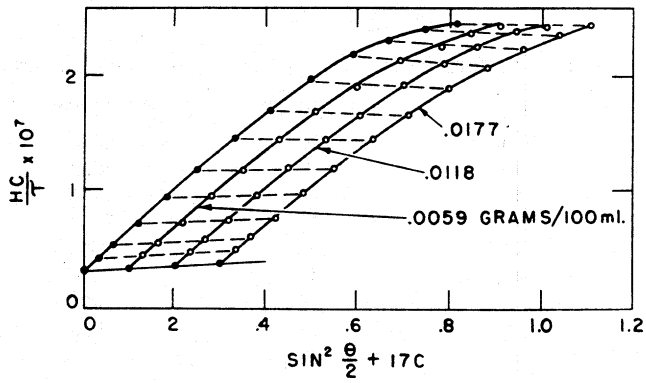

Fig. 1. Bilinear plot of the light scattering data for amylopectin I in water.

experimental data are shown as open circles and the extrapolated points as solid circles. Lines of constant concentration were extrapolated linearly from $\theta = 60°$ downward to zero angle A line through the resulting points intercepts the vertical axis at an Hc/τ value corresponding to a molecular weight of 35×10^6. As shown in Figure 1, extrapolation of the lines of constant angle to zero concentration, followed by an extrapolation to zero angle, gives an identical intercept and molecular weight value. Similar extrapolations of the data obtained at wave length 546 mμ yielded a value of 33×10^6 for the molecular weight. The absence of marked convex-upward curvature in the low-angle region of the lines of constant concentration indicates the absence of large particles of dirt or microgel in solution.

It has been shown[7,18] that a particle dimension can be calculated from the ratio of the initial slope to the intercept of the angular scattering curve without any assumptions concerning particle shape. The radius of gyration, or root-mean-square radius from the center of mass of the particle, is obtained from the initial slope and intercept of the angular scattering curve by the following relationship:

$$\frac{\text{Initial slope}}{\text{Intercept}} = \frac{16\pi^2}{3}\frac{R_g^2}{\lambda^2}$$

where R_g^2 is the mean-square radius of the particle, and λ is the wave length of the incident light in the solution. For a polydisperse sample, R_g^2 is an average over the distribution of molecular sizes. For randomly coiled linear chains, Zimm[18] has shown that the average entering the slope from which the characteristic length is determined is the Z-average; the average depends on the molecular model[19] but is always greater than a weight-average if the radius increases with molecular weight. For a branched chain polymer it can be shown that the square of the unit segment length is weighted by the Z-average molecular weight as in the case of a linear polymer. The radius of gyration of amylopectin I was 1950 and 1910 A. for wave lengths of 436 and 546 mμ, respectively.

TABLE I

LIGHT SCATTERING CHARACTERIZATION OF FRACTIONS OF AMYLOPECTIN I

Fraction	Weight, %	Mol. wt. $\times 10^{-6}$	Radius of gyration, A.
1	5	73	2550
2	10	73	2550
3	16	47	1950
4	8	31	1660
5	13	23	1370
6	16	19	1250
7	8	14	1090
8	8	11	960
9	11	7	820

For three separate determinations on amylopectin I the average value of the molecular weight was $36 \times 10^6 \pm 4 \times 10^6$, and the average radius was 1880 ± 80 A. Molecular weights as reported do not include a correction for depolarization. The measured depolarization for the amylopectin was approximately 2%, signifying that the optical anisotropy of its molecules in solution was small.

Effect of Temperature and Alkali on the Light Scattering of Amylopectin I. To investigate the effect of temperature on the scattering of dilute solutions of amylopectin, a 0.02% solution was heated in an oven to 90°C., and the light scattered at 90° to the incident beam was measured in the temperature range 75 to 25°. No change in the scattering was observed. To eliminate the possibility of a compensating refraction change, the refractive index and refractive increment were measured as a function of temperature. There was less than a 1% increase in refractive increment on raising the temperature from 25 to 75°, and less than a 1% decrease of the refractive index. The observation that the fraction of light scattered was unaffected by temperature can be interpreted, therefore, as evidence that the average weight of the amylopectin particles did not change.

Alkali is generally considered to be a good solvent for starch and is often resorted to when preparations are found difficult to disperse in water. MacMillan and Melvin[20] have demonstrated that whereas amylose is rapidly degraded in heated solutions of alkali, amylopectin is relatively resistant to attack. An amylopectin solution, 0.5 *N* sodium hydroxide, was heated for 2 hours at 90° in the absence of oxygen.[20] The decrease in the scattering of the solution in the 90° direction was less than 10%. Similar results were obtained when a water solution was heated for 1 hour at 120° in a sealed tube free from oxygen. These results indicate that if the particles in solution are aggregates, they are not broken up by temperature or alkali.

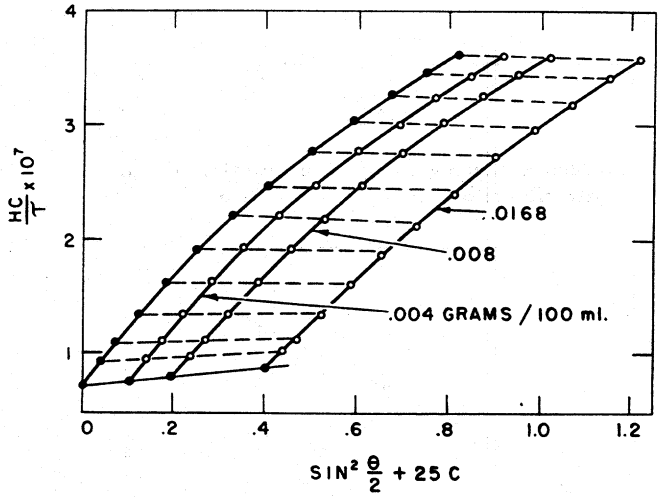

Fig. 2. Bilinear plot of the light scattering data for amylopectin II in water.

Measurements on Amylopectin I Fractions. The angular distribution of the intensity of scattered light for each of the nine fractions of amylopectin I was determined in the manner described for the unfractionated material, and the results are summarized in Table I. Molecular weights of the fractions ranged from 73 to 7 million, with one-third of the amylopectin having a molecular weight of 48 to 73 million. The weight-average molecular weight for the nine fractions was 36 million, in good agreement with the value, 36 million, found for the unfractionated sample. Radii of gyration of the fractions ranged from 820 to 2550 A. The interaction constant, B, calculated from the concentration dependence, ranged from 6×10^{-6} for Fraction 1, having molecular weight 73 million, to 44×10^{-6} cc. moles/g.^{2} for Fraction 9, having 7 million molecular weight.

Light Scattering of Water Solutions of Amylopectin II. The angular distribution of scattering given by one set of measurements at wave length 436 mμ on amylopectin II is presented in Figure 2. Values of

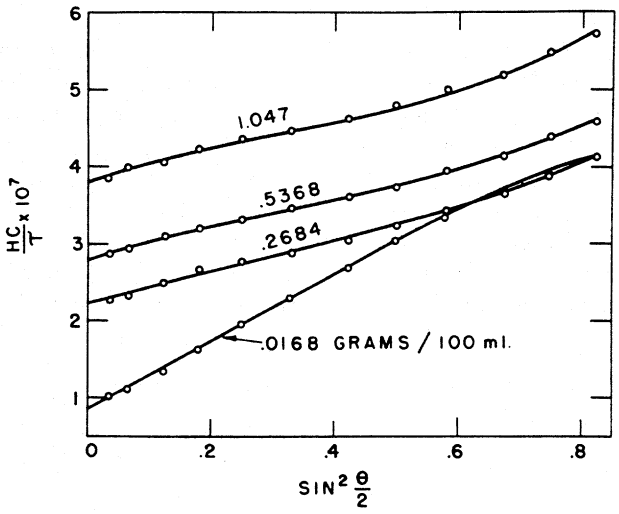

Fig. 3. Angular scattering curves as a function of concentration of amylopectin II in water.

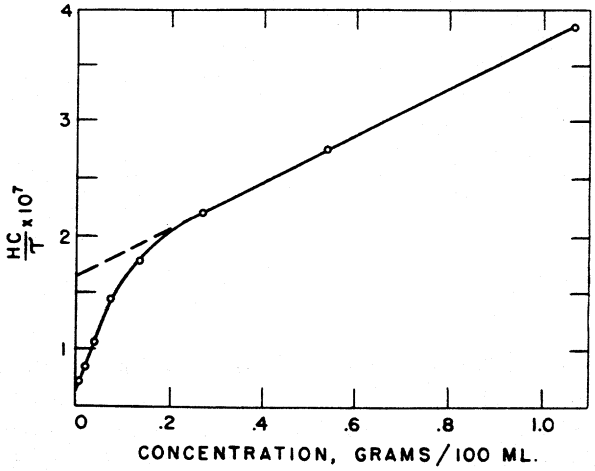

Fig. 4. Reciprocal specific turbidity values for amylopectin II in water solution as a function of concentration.

the molecular weight ranged from 13.2 to 14.9 million for four separate determinations, with an average of 14 million. The average value for the radius of gyration was 1090 A.

The importance of measurements at low amylopectin concentrations is shown by the data of Figures 3 and 4. As illustrated in Figure 3, the slope of the angular scattering curve was markedly reduced at concentrations of 0.26 g./100 ml. and above. Extrapolated values of Hc/τ from Figure 3 are plotted in Figure 4 as a function of concentration. It can be seen that extrapolation of the upper branch of this curve as determined by concentra-

tions above 0.3 g./100 ml. results in a much lower molecular weight, 6.3 million, than that obtained from the data at lower concentrations. Comparison of the angular scattering curves at 0.0168 g./100 ml. and 0.2684 g./100 ml. of Figure 3 shows that a major effect of the higher concentration has been the diminution of scattering intensity at forward angles compared to the symmetrical backward direction. This phenomenon has been observed by Doty and Steiner[21] in the case of certain macromolecular polyelectrolytes and proteins in salt-free solution. An analogous effect occurs in x-ray scattering[22] and has been interpreted to arise from the finite size and/or electrostatic repulsions of particles which prevents the attainment of a completely random array of scattering centers.

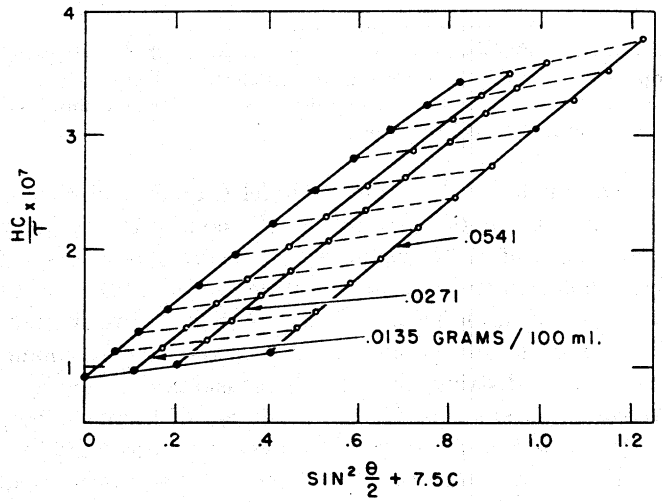

Fig. 5. Bilinear plot of the light scattering data for amylopectin II in 0.2 *N* NaOH.

Scattering measurements also were made on solutions of amylopectin II in 0.2 *N* NaOH. From the data in Figure 5, a value of 11 million was obtained for the molecular weight, which was somewhat lower than the value in water solutions. It should be mentioned that the refractive index increment of amylopectin in 0.2 *N* NaOH solution was 0.142 ml./g. at 436 mμ. This is considerably lower than the value 0.156 found for water solutions, and is also slightly less than the value 0.146 found by Paschall and Foster[23] for amylose in both neutral KCl solution and in 1 *M* KOH.

Compared to the value, 1090 A., found for the radius of gyration in water solution, a value of 840 A. was measured in 0.2 *N* NaOH. The contraction of the amylopectin molecule in alkali may partially result from a neutralization of the effect of the charge of the phosphate groups by the ionic environment provided by the alkali solution. As will be described later, a similar effect occurs on the addition of neutral electrolytes.

Polydispersity of Amylopectin II and Fraction 4 of Amylopectin I. Evidence for the polydispersity of amylopectin II was obtained from

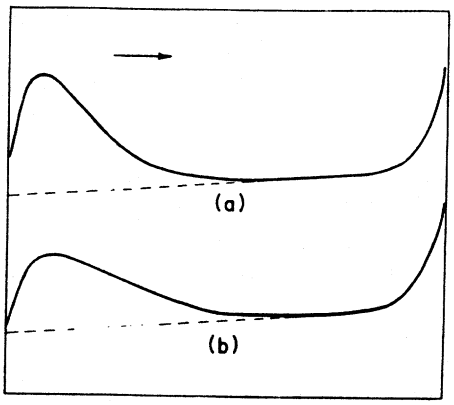

Fig. 6. Tracings of sedimentation diagrams of a 0.14% solution of amylopectin II: (a) 12 minutes and (b) 20 minutes after reaching a speed of 37,020 r.p.m. Arrow indicates direction of sedimentation and dotted lines are extensions of base lines.

sedimentation diagrams taken in a Spinco Model E analytical ultracentrifuge. Amylopectin was centrifuged at 0.14% concentration in water solution at a rotor speed of 37,020 r.p.m. and exposures taken at 4-minute intervals. Figure 6 is a tracing of sedimentation patterns taken at 12 and 20 minutes after centrifugation began. The breadth of the peaks indicates a wide distribution of sedimentation constants and a correspondingly broad molecular weight distribution. From measurements on the sedimentation patterns, the sedimentation constant, S_{20}, of the most frequent species was found to be 8.8×10^{-13}. However, the most rapidly sedimenting species had an S_{20} value of 40.3×10^{-13}. For comparison, the sedimentation constant of the most frequent species in Fraction 4 of amylopectin I was 44.8×10^{-13} at 0.15% concentration. It must be noted that these values were determined at relatively high concentrations, and measurements at lower concentrations would be expected to give larger values. The effect of concentration was clearly shown in the case of Fraction 4, for which S_{20} was found to be 29.4×10^{-13} in 0.30% solution. A measure of the distribution of sedimentation constants of Fraction 4 and, thus, its polydispersity is provided by values of dB/dx calculated according to Gralén.[24] At 0.30 and 0.15% concentration, these values were 0.59 and 0.50, respectively.

To investigate the possibility that a small fraction of disproportionately large aggregates might be present in the amylopectin solution, careful observation of the sedimentation pattern was made during the acceleration of the rotor and the early periods of centrifugation. No indication of a very rapidly sedimenting component was observed.

Molecular Weight and Dimensions of the Partial Acetate of Amylopectin II in Organic Solvents. Light scattering measurements were made on the partial acetate of amylopectin II dissolved in acetone, chloroform, dioxane, nitromethane, and acetonitrile. Because of the less

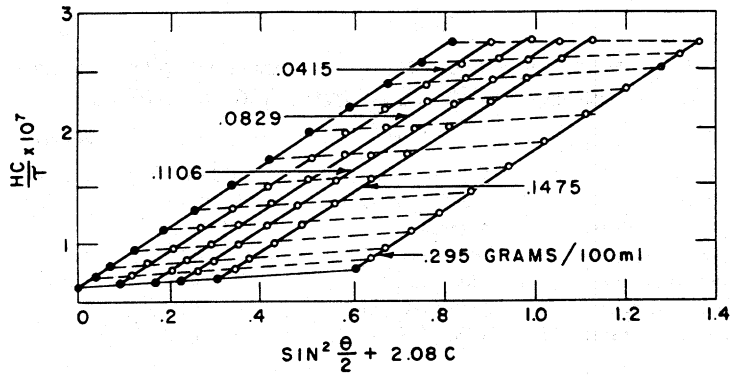

Fig. 7. Bilinear plot of light scattering data for amylopectin II acetate in chloroform.

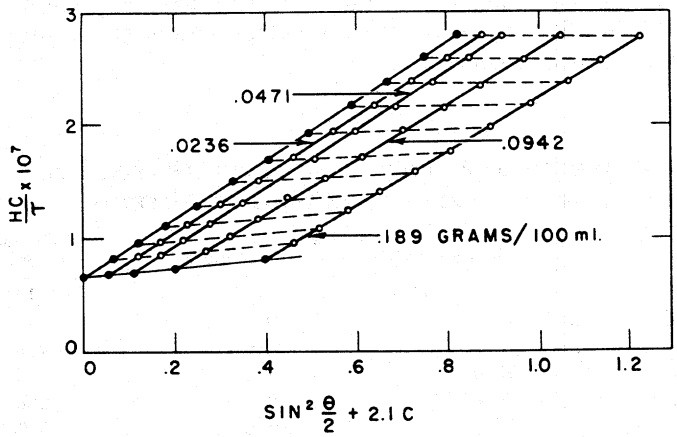

Fig. 8. Bilinear plot of light scattering data for amylopectin II acetate in nitromethane.

favorable refractive index increments (Table II) of the acetate in the organic solvents, higher concentrations than those used in water solution were necessary to produce turbidities in the desired range. Zimm plots of the light scattering data for solutions in chloroform and nitromethane are presented in Figures 7 and 8, respectively.

All measurements of the molecular weight of the amylopectin acetate were in the range 15 to 18 million, as shown in Table III. Radii of gyration of the particles varied from 1010 to 860 A. in the five organic solvents. Included in Table III are the results on a partial acetate which had not been precipitated from its acetylation mixture. Instead, the mixture was dialyzed against acetone for three days, thereby minimizing any tendency to form undispersible aggregates which might result from precipitation. The light scattering molecular weight, after clarification of the solution, was 17.4 million, compared with 18 million for the precipitated preparation dissolved in acetone. By correcting for the acetyl content, molecular

TABLE II

REFRACTIVE INCREMENT OF AMYLOPECTIN AND AMYLOPECTIN ACETATE IN VARIOUS SOLVENTS AT WAVE LENGTHS 436 AND 546 mμ

Substance	Solvent	436 mμ	546 mμ
Amylopectin	Water	0.156	0.154
Amylopectin	0.2 *N* NaOH	0.142	—
Amylopectin acetate	Acetone	0.118	—
Amylopectin acetate	Dioxane	0.057	—
Amylopectin acetate	Chloroform	0.051	—
Amylopectin acetate	Nitromethane	0.088	—
Amylopectin acetate	Acetonitrile	0.128	—

weight values for amylopectin can be calculated from the molecular weight of the acetate, and these values are given in column 3 of Table III. The molecular weight for amylopectin II obtained in this manner was about 10 million, which is in fair agreement with the values obtained for water and alkali solutions of the amylopectin.

TABLE III

MOLECULAR WEIGHT OF AMYLOPECTIN II ACETATE FROM LIGHT SCATTERING MEASUREMENTS IN ORGANIC SOLVENTS

Solvent	Molecular weight		Radius of gyration, A.
	Amylopectin acetate	Amylopectin	
Acetone	18.0×10^6	10.8×10^6	1010
Chloroform	15.9	9.5	930
Dioxane	15.5	9.6	940
Nitromethane	15.4	9.5	870
Acetonitrile	15.6	9.7	860
Acetone[a]	17.4	10.4	990

[a] Amylopectin acetate solution prepared by dialysis of acetylation reaction mixture against acetone.

Deacetylation of the Partial Acetate of Amylopectin II. To further test the stability of the amylopectin particles, amylopectin acetate was deacetylated in 0.2 *N* NaOH and the molecular weight of the resulting amylopectin was determined. After three days at room temperature, 2 grams of the partial acetate dissolved completely in 100 ml. of 0.2 *N* NaOH. The resulting solution was dialyzed against distilled water to remove the

TABLE IV

MOLECULAR WEIGHT AND LIGHT SCATTERING RADIUS OF AMYLOPECTIN II AND ITS ACETATE

	Molecular weight	Radius of gyration, A.
Before acetylation	14.0×10^6	1090
From acetate (average value)	9.9	—
Deacetylated	9.4	980

salt, and light scattering measurements were made on the clarified solution. The molecular weight of the amylopectin was 9.4 million, which is in good agreement with the values obtained for this amylopectin prior to acetylation. Comparison of the results given in Table IV indicate that the molecules of amylopectin II were stable in various solvents and only slightly degraded by mild chemical treatment in the formation and hydrolysis of the acetate.

Effect of Electrolyte on the Scattering of Amylopectin II. The polyelectrolyte character of potato amylopectin should be reflected in a change of extension or configuration of the molecule when the negative charges of its phosphate groups are shielded by the addition of electrolyte. This change in configuration should result in a change in the radius of gyration as measured by the light scattering method.

The angular distribution of light scattered from water solutions of amylopectin II was measured at 3 amylopectin concentrations and at 5 molarities of potassium chloride in the range 1×10^{-5} to 5×10^{-3}. The molecular weight, radius of gyration, and interaction constant at the various molarities of added electrolyte are given in Table V. There was a decrease in the particle radius, but little change in particle weight of the amylopectin with increasing potassium chloride concentration up to a concentration of 5×10^{-4} *M*. Concurrent with the decrease in particle radius, there was a decrease in the interaction constant, B, as the ionic strength of the solutions increased. At a potassium chloride concentration of 1×10^{-3} *M*, however, there was an increase in both the particle weight and particle radius, indicating onset of aggregation of the amylopectin molecules. At concentrations of potassium chloride above 5×10^{-3} *M*, the amylopectin aggregated to such an extent that the solutions could no longer be passed through ultrafine sintered-glass filters, and light scattering measurements were discontinued. It is interesting to note that the minimum particle radius of the amylopectin molecule in the presence of potassium chloride was about that found for amylopectin in 0.2 *N* sodium hydroxide solutions. There is the important difference in these solutions, however, that amylopectin is stable toward aggregation in alkali solutions, whereas

TABLE V

EFFECT OF POTASSIUM CHLORIDE ON THE SCATTERING OF WATER SOLUTIONS OF AMYLOPECTIN II

KCl, molarity	Molecular weight	Radius of gyration, A.	Interaction constant, B
0	13.6×10^6	1010	0.52×10^{-4}
0	13.4	975	0.48
1×10^{-5}	13.2	936	0.41
1×10^{-4}	13.9	940	0
5×10^{-4}	14.1	890	0
1×10^{-3}	19.6	1040	0
5×10^{-3}	19.2	960	0

it aggregates rapidly in the former at concentrations above 10^{-3} M potassium chloride.

DISCUSSION

The present results indicate that the molecular weight of potato amylopectin is greater than 10 million, and that this value may be dependent on the fractionation treatment or the original starch, as evidenced by the values 36×10^6 and 14×10^6 found for amylopectins I and II, respectively. Separation of amylopectin I into nine fractions, ranging in weight-average molecular weight from 7 to 73 million, gives a measure of the polydispersity of this preparation. Assuming these fractions to be monodisperse, the number-average molecular weight of amylopectin I was calculated to be 18 million. This represents an upper limit for M_n since the fractions cannot be expected to be monodisperse. The ultracentrifuge patterns of Fraction 4 show that a distribution of sizes is present and, based on a comparison with patterns of high polymers of similar nature, the ratio of weight to number-average values for Fraction 4 would be 1.5 to 2. Applying this factor to all fractions would give a better estimate for number-average molecular weight. Although no quantitative analysis of the molecular weight distribution of amylopectin II has been made, the distribution of sedimentation constants determined in the ultracentrifuge indicates an even broader molecular weight distribution than that found for amylopectin I. Assuming similar polydispersity for the amylopectins measured by Potter and Hassid,[1] the values (1 to 6 million) they report for their samples of wheat, sago, Easter lily, corn, and tapioca amylopectins from osmotic pressure measurements on the acetylated derivatives are comparable to our value for amylopectin II. Discrepancies between light scattering and osmotic pressure values for amylopectin would be magnified, of course, if the samples contained an appreciable fraction of amylose as an impurity, for this relatively low molecular weight[1,16] component would reduce the number-average molecular weight much more than the weight-average.

No evidence was found for an increase in reciprocal specific turbidity at low concentrations which would parallel the observations of Kerr *et al.*[2] on the osmotic pressure of corn amylopectin acetate in chloroform. These authors found that the specific osmotic pressure, π/c, reached a minimum around 0.3% concentration and then increased on going to 0.1% concentration, the lower limit of their measurements. No such abnormality was observed in the reciprocal specific turbidity plot of potato amylopectin acetate in chloroform (Fig. 7), nitromethane (Fig. 8), dioxane, acetonitrile, or acetone, nor for amylopectin in water or alkali solutions (Figs. 1, 2, and 5). On the contrary, greater slope at low concentrations was observed at low concentrations of amylopectin II in water solution as illustrated by Figure 4.

Comparison of the molecular weight values for amylopectin II in aqueous solution (Table IV) with those of its partial acetate in organic solvents (Table III) supports a value of at least 10 to 14 million for the average

weight of the smallest kinetic unit of this amylopectin. It is possible that the lower value, 10 million, calculated from the molecular weight of the acetate in organic solvents, may have resulted from disaggregation of the 14 million average unit found in water solutions. Degradation during acetylation may be responsible, however, and we have no evidence that covering the hydroxyl groups through acetylation permits the dissociation of potato amylopectin into smaller units.

Similarly, 36 million appears to be the average weight of the smallest kinetic unit of amylopectin I since conditions favorable to dissociation of aggregates did not alter this value significantly. These included autoclaving water solutions for 1 hour at 120°; heating oxygen-free solutions, 0.5 *N* in NaOH, for 2 hours at 90°; and the direct measurement of the scattering from water solutions at elevated temperatures. Zimm and Thurmond[25] reported a molecular weight of 420,000,000 for an acetylated Easter lily amylopectin from light scattering measurements. Likewise, the particles did not dissociate on heating.

Certain preparative treatments, however, have been found to reduce the molecular weight of amylopectin. In particular, agitation of a 1% solution of amylopectin II for 30 minutes in a Waring Blendor in the presence of air reduced its molecular weight to 5 million. Degradation of amylopectin resulting from the shearing action of the blendor has been postulated previously[24] on the basis of intrinsic viscosity measurements. Confirmation that the shearing action of velocity gradients in solutions of high molecular weight polymers can break primary bonds has been demonstrated recently by the results on polystyrene solutions forced through small orifices at high velocity.[27] The differences in molecular weight of amylopectins I and II cannot be accounted for on the basis of mechanical degradation since amylopectin I, having the higher molecular weight, was dispersed with the aid of a "semicolloid mill." The shearing action of this mixer is much lower, however, than that of the Waring Blendor. It appears more likely that the lower molecular weight of amylopectin II resulted either from previous treatment of the parent starch or from inadvertent chemical degradation during the fractionation. In this connection it should be remarked that there are sedimentation data which indicate that potato amylopectin may have a considerably greater molecular weight than that reported for our preparation I. Horan[3] has reported a value of 65 *S* for the sedimentation constant of a potato amylopectin determined from measurements in the concentration range 0.38 to 0.93 g./100 g. in water solution. This may be compared with the sedimentation constant, 29.4 *S*, found at 0.30% concentration for Fraction 4 of amylopectin I having a molecular weight of 31 million. Thus, further investigations of different starch preparations and fractionation treatments are indicated.

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Synopsis

Light scattering measurements were made on amylopectin prepared from potato starch by two procedures. Potato starch dispersed in water at 90° and fractionated with mixed pentanols gave an amylopectin I having a weight-average molecular weight of 36 million. Dispersion of starch by autoclaving at 120°, followed by nitrobenzene precipitation of amylose, yielded an amylopectin II with weight-average molecular weight of 14 million. No evidence was found for dissociation of these amylopectins into smaller units in aqueous solution at concentrations as low as 10^{-6} g./ml., at elevated temperatures, or in sodium hydroxide solutions. Aggregation of amylopectin II occurred at potassium chloride concentrations above 5×10^{-4} molar. Marked reduction of molecular weight of amylopectin II resulted from treatment under a high rate of shear in a Waring Blendor. Acetylated II (40% acetyl content) was soluble in nitromethane, chloroform, acetone, dioxane, and acetonitrile. In all these solvents the molecular weight, corrected for acetyl, was close to 10 million. No evidence was found for an increase in reciprocal specific turbidity at low concentration which would parallel reported osmotic pressure results. Polydispersity was demonstrated by ultracentrifugal

sedimentation patterns of amylopectin II, and by separation of amylopectin I into a series of fractions ranging from 7 to 73 million in molecular weight.

Résumé

Des mesures de diffusion de lumière ont été effectuées sur l'amylopectine d'amidon de pommes de terre obtenu par deux méthodes. L'amidon de pommes de terre dispersé dans l'eau à 90° et fractionné avec un mélange de pentanols fournit une amylopectine I dont le poids moléculaire atteint 36 millions. La dispersion de l'amidon à l'autoclave à 120°C, suivie d'une précipitation de l'amylose au nitrobenzène, fournit à son tour une amylopectine II dont le poids moléculaire atteint 14 millions. On n'a obtenu aucune preuve de la dissociation de ces amylopectines en unités plus petites en milieu aqueux même aux concentrations les plus faibles, telles que 10^{-6} g./ml. et à température élevée; il en est de même en soluton dans la soude caustique. L'aggrégation de l'amylopectine II se passe en présence de concentrations en chlorure de potassium plus élevée que $5 \times 10^{-4}M$. On observe par ailleurs une forte diminution de poids moléculaire de l'amylopectine II en la traitant à vitesse élevée dans un "Mixer." Le produit II acétylé (40% de teneur en fonctions acétyles) est soluble dans le nitrométhane, le chloroforme, l'acétone; le dioxane et l'acétonitrile. Dans tous ces solvants le poids moléculaire, corrigé pour sa teneur en fonctions acétyles, se situe aux environs de 10 millions. On n'a pas observé d'augmentation de l'inverse de la turbidité spécifique aux faibkes concentrations, phénomène qui a été observé dans les mesures osmotiques antérieures. On a pu démontrer la polydispersité de l'amylopectine II par des diagrammes de sédimentation à l'ultracentrifuge; dans le cas de l'amylopectine I on a pu le démontrer par la séparation de fractions dont les poids moléculaires variaient de 7 à 73 millions.

Zusammenfassung

Es wurden Lichstreuungsmessungen an Amylopektin ausgeführt, welches aus Kartoffelstärke mittels zwei Methoden hergestellt wurde. Kartoffelstärke, welche in Wasser bei 90° dispergiert und mit Pentanolgemischen fraktioniert wurde, ergab ein Amylopektin I, welches ein Gewichtsmittel-Molekulargewicht von 36 Millionen hatte. Dispersion von Stärke im Autoklaven bei 120° und Fällung der Amylose durch Nitrobenzol ergab ein Amylopektin II mit einem Gewichtsmittel-Molekulargewicht von 14 Millionen. Es wurde keine Evidenz von Dissoziation dieser Amylopektine in kleinere Einheiten in wässriger Lösung bei Konzentrationen bis auf 10^{-6} g./ml. herab gefunden, auch nicht bei erhöhten Temperaturen oder in Natriumhydroxydlösungen. Aggregation von Amylopektin II trat bei Kaliumchloridkonzentrationen über $5 \times 10^{-4}M$ auf. Eine erhebliche Reduktion des Molekulargewichtes von Amylopektin II wurde durch Behandlung im Waring Blender bei einer hohen Schubgeschwindigkeit hervorgerufen. Acetyliertes II (40% Acetylgehalt) war in Nitromethan, Chloroform, Aceton, Dioxan und Acetonitril löslich. In all diesen Lösungsmittels war das für Acetyl korrigierte Molekulargewicht nahezu 10 Millionen. Es wurde keine Evidenz für eine Zunahme der reziproken spezifischen Trübung bei niedriger Konzentration gefunden, welche parallel zu berichteten osmotischen Druck-Resultaten sein würde. Polydispersität wurde durch ultrazentrifugale Sedimentationsdiagramme von Amylopektin II, und durch Separation von Amylopektin I in eine Reihe von Fraktionen mit Molekulargewichten im Bereich von 7 bis 73 Millionen gezeigt.